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Molecular cloning of human cardiac troponin I using polymerase chain reaction.

Vallins WJ, Brand NJ, Dabhade N, Butler-Browne G, Yacoub MH, Barton PJ.

Department of Cardiothoracic Surgery, National Heart and Lung Institute, London, UK.

We have used the polymerase chain reaction (PCR) to synthesise a cDNA encoding part of human cardiac troponin I. Amplification was achieved using fully degenerate sets of oligonucleotides corresponding to conserved regions of amino acid sequence identified in other troponin I isoforms. The cloned PCR fragment was subsequently used to isolate full-length cDNAs from a cardiac cDNA library. We describe the approach, as a general cloning strategy starting from limited amino-acid sequence data and report the cloning, and complete amino acid sequence of human cardiac troponin I. Analysis of human development using these clones demonstrates early expression of this gene in the heart.

PMID: 2226790 [PubMed - indexed for MEDLINE]

1: FEBS Lett 1999 Jun 18;453(1-2):107-12 Related Articles Books LinkOut

NMR analysis of cardiac troponin C-troponin I complexes: effects of phosphorylation.

Finley N, Abbott MB, Abusamhadneh E, Gaponenko V, Dong W, Gasmi-Seabrook G, Howarth JW, Rance M, Solaro RJ, Cheung HC, Rosevear PR.

Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, College of Medicine, OH 45267, USA.

Phosphorylation of the cardiac specific amino-terminus of troponin I has been demonstrated to reduce the Ca2+ affinity of the cardiac troponin C regulatory site. Recombinant N-terminal cardiac troponin I protein, cardiac troponin I(33-80), cardiac troponin I(1-80), cardiac troponin I(1-80)DD and cardiac troponin I(1-80) pp, phosphorylated by protein kinase A, were used to form stable binary complexes with recombinant cardiac troponin C. Cardiac troponin I(1-80)DD, having phosphorylated Ser residues mutated to Asp, provided a stable mimetic of the phosphorylated state. In all complexes, the N-terminal domain of cardiac troponin I primarily makes contact with the C-terminal domain of cardiac troponin C. The nonphosphorylated cardiac specific amino-terminus, cardiac troponin I(1-80), was found to make additional interactions with the N-terminal domain of cardiac troponin C.

PMID: 10403385 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 2002 Jul 1;306(1):92-9 Related Articles, Books, LinkOut

FULL-TEXT ARTICLE

Separation of synthetic oligonucleotide dithioates from monothiophosphate impurities by anion-exchange chromatography on a mono-q column.

Yang X, Hodge RP, Luxon BA, Shope R, Gorenstein DG.

Sealy Center for Structural Biology

A method using a strong anion-exchange liquid-chromatography column, Mono-Q, has been developed for high-resolution analysis and purification of oligonucleotide dithioates, which were synthesized by an automated, solid-phase, phosphorothioamidite chemistry. High-resolution separation of oligonucleotide phosphorodithioates from monothiophosphate impurities was obtained. High-resolution separation was also demonstrated at pH 8. The separation of oligonucleotide dithioates was found to be linearly dependent on the number of sulfurs for the same sequence length. Thiocyanate, SCN(-), as eluting anion, can be used to purify oligonucleotides containing a high percentage of phosphorodithioate linkages in lower salt concentrations and provides better separation than chloride as eluting anion. (c) 2002 Elsevier Science (USA).

PMID: 12069419 [PubMed - in process]

Modified aluminas as chromatographic supports for high-performance liquid chromatography.

Pesek JJ, Matyska MT.

Department of Chemistry, San Jose State University, College of Science, CA 95192-0101, USA.

This review begins by describing the relevant properties of alumina as a support material for chemically bonded stationary phases in HPLC. The most common chemical modification processes are summarized as well as the advantages and disadvantages of each method. In order to more fully understand the chemically modified alumina surface, some spectroscopic approaches are outlined for characterization of the bonded phases. Finally, a number of successful applications are described for a variety of chemically modified aluminas in order to illustrate their potential usefulness and to compare their chromatographic behavior to the more conventional silica-based materials.

PMID: 12064521 [PubMed - in process]

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Application of reversed phase high performance liquid chromatography for subproteomic analysis of cardiac muscle.

Neverova I, Van Eyk JE.

Department of Physiology, Queen's University, Kingston, ON, Canada.

The application of protein separation methodologies, such as reversed phase chromatography, should allow differential separation of the proteome, or at least specific subproteomes, comparable to that achieved by two-dimensional electrophoresis (2-DE). A rapid sequential protein extraction method (termed "IN Sequence") was developed to isolate three distinct subproteomes of cardiac muscle. Two subproteomes, those enriched for the cytoplasmic or myofilament proteins, can be separated by either reversed phase high performance liquid chromatography (RP-HPLC) or 2-DE. Reversed phase HPLC of the myofilament protein enriched extract was optimized for resolution and peak numbers by altering flow rate, gradient rate and the organic modifiers, isopropanol and acetronitrile. The myofilament protein enriched extract from failing swine heart, due to coronary artery ligation (LAD), was compared to the extract from a sham operated animal (SHAM). The HPLC chromatograms of these extracts were similar, but distinctive in many regions. The HPLC fractions, collected within some of these distinct regions of the chromatograms were analyzed using peptide mass fingerprinting - mass spectrometry and immunoblot analysis. Two myofilament proteins, troponin T and myosin heavy chain, were identified and found differentially modified in the SHAM and LAD hearts. Both troponin T and myosin heavy chain are problematic proteins for 2-DE, but yet they were resolved by reversed phase chromatography. Therefore, RP-HPLC can be used in conjunction with 2-DE to enhance protein separation of myofilament protein subproteome.

PMID: 11788988 [PubMed - indexed for MEDLINE]

1: Eur J Biochem 1994 Nov 1;225(3):1195-201 Related Articles, Books, LinkOut

Overexpression of human cardiac troponin-I and troponin-C in Escherichia coli and their purification and characterisation. Two point mutations allow high-level expression of troponin-I.

al-Hillawi E, Minchin SD, Trayer IP.

School of Biochemistry, University of Birmingham, England.

We have overexpressed human cardiac troponin-I in Escherichia coli. Initially, protein expression was not detected in the bacterial cell extracts. Systematic deletion of the N-terminal region of the protein generated a series of truncated mutants which were expressed at varying levels in the bacteria. This allowed us to narrow the problem down to the first five codons in the gene sequence. In order to achieve expression at high levels, two base changes were required, in the second and the fourth codons of the cDNA sequence. The codon changes, (Ala2) GCG-->GCC and (Gly4) GGG-->GGT, do not alter the coding potential of the DNA. We have also overexpressed the human cardiac isoform of troponin-C. Both proteins were purified using ion-exchange chromatography and have been proved to be biologically active. The recombinant troponin-I was able to bind to a troponin-C affinity column in the presence of 9 M urea in a calcium-dependent manner. The calcium-dependent troponin-I-troponin-C complex between both recombinant proteins was also demonstrated by alkaline-urea gel electrophoresis. In addition, troponin-I inhibited the acto-S1 Mg-ATPase activity; this inhibition was potentiated by the presence of tropomyosin and was reversed by the addition of troponin-C to the system. Biological activity was also demonstrated in vivo in that the recombinant proteins were able to restore the calcium-dependent force generation to calcium-insensitive skinned muscle fibres.

PMID: 7957210 [PubMed - indexed for MEDLINE]

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Overexpression of human cardiac troponin-I and troponin-C in Escherichia coli and their purification and characterisation. Two point mutations allow high-level expression of troponin-I.
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Molecular cloning of human cardiac troponin I using polymerase chain reaction.
FEBS Lett. 1990 Sep 17;270(1-2):57-61.
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Reconstitution of rabbit skeletal muscle troponin from the recombinant subunits all expressed in and purified from E. coli.

Fujita-Becker S, Kluwe L, Miegel A, Maeda K, Maeda Y.

European Molecular Biology Laboratory, Desy, Hamburg, Germany.

Three subunits of rabbit skeletal muscle troponin were expressed in and purified from Escherichia coli. The procedures were optimized, and the reconstituted troponin complex is highly homogeneous, stable, and obtainable in large quantities, allowing us to conduct crystallization studies of the troponin complex. The three subunits expressed and purified are beta-TnT(N'-208), TnI(C64A, C133S), and the wild type TnC. beta-TnT(N'-208) is a 25 kDa fragment of beta-troponin T, which consists of 208 amino acids and lacks 58 residues in the N-terminal variable region. TnI(C64A, C133S) is a mutant troponin I, in which Cys-64 and Cys-133 are replaced by Ala and Ser, respectively. Each subunit was separately expressed in E. coli, purified by column chromatography including HPLC, and reassembled to form troponin complex. The reconstituted troponin complex was not distinguishable from authentic troponin prepared from rabbit skeletal muscle: the acto-S1 ATPase rate, as well as the superprecipitation, was calcium-sensitive. Small flat crystals up to 0.2 mm long have been reproducibly obtained in preliminary crystallization trials.

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